CLAIMS

What is claimed is:

1. A method for detecting low frequency mutations in a target sequence from a DNA sample comprising the steps of:

a) enriching a DNA sample for one or more target sequences, wherein the enrichment step comprises sequence-specific hybridization to the target sequences with one or more labeled probes, wherein each labeled probe is complementary to a specific target sequence, resulting in about a 10^3-fold to about a 10^4-fold enrichment of target sequences from the DNA sample thereby obtaining a target-enriched sample; and

b) detecting mutations in the target sequence or sequences from the target-enriched sample.

2. The method of Claim 1, wherein the enrichment step comprises:

a) denaturing double-stranded DNA;

b) contacting the denatured DNA with one or more probes comprising a sequence complementary to one or more target sequences to form a mixture;

c) maintaining the mixture of step b) under conditions such that probe-fragment hybrid molecules are formed; and

d) isolating the probe-fragment hybrids from the mixture,

resulting in a target-enriched sample and a depleted sample.

3. The method of Claim 2, wherein the DNA sample fragmented prior to denaturation.

4. The method of Claim 1, wherein a probe complementary to a specific target sequence comprises an affinity moiety unique for a specific target sequence.

5. The method of Claim 1, wherein a plurality of target sequences is concurrently enriched from a sample resulting in a plurality of target-enriched DNA samples.

6. The method of Claim 2, wherein the depleted sample is subjected to a subsequent enrichment step to enrich for one or more target sequences different from the target sequences obtained in the first enrichment step.

7. The method of Claim 1, wherein the DNA sample comprises single-stranded DNA molecules.

8. The method of Claim 1, wherein the DNA sample comprises double-stranded molecules.

9. The method of Claim 1, wherein constant denaturant capillary electrophoresis is used to detect nuclear mutations.

10. The method of Claim 1, wherein allele-specific polymerase chain reaction is used to detect nuclear mutations.

11. The method of Claim 1, wherein the mutation detected is present at a mutant fraction about or higher than 10^{-6}.

12. A method for detecting low frequency nuclear mutations in a target sequence from a genomic DNA sample comprising the steps of:

a) enriching the DNA sample for molecules comprising one or more target sequences, thereby preparing a target-enriched sample comprising mutant and non-mutant sequences, wherein the enrichment step comprises sequence-specific hybridization with one or more labeled probes that hybridize to the target sequences resulting in about a 10^3-fold to about 10^4-

fold enrichment of molecules comprising target sequences from the DNA sample;

b) subjecting the target-enriched sample to constant denaturant capillary electrophoresis using a wide-bore capillary to separate mutant heteroduplexes from non-mutant homoduplexes;

c) amplifying the heteroduplexes of step b) by high fidelity polymerase chain reaction to obtain amplified polymerase chain reaction products;

d) subjecting the polymerase chain reaction products of step c) to constant denaturant capillary electrophoresis to further enrich the sample for mutants, thereby creating a mutant-enriched sample;

e) subjecting the mutant-enriched sample of step d) to constant denaturant capillary electrophoresis to obtain a mutational spectra; and

f) selecting one or more individual mutant fractions from the mutational spectra for sequence analysis to detect mutations.

13. The method of Claim 12, wherein step b) further comprises subjecting mutant heteroduplexes to capillary electrophoresis prior to high fidelity polymerase chain reaction.

14. The method of Claim 12, wherein step d) further comprises subjecting the target-enriched sample to one or more additional rounds of constant denaturant capillary electrophoresis prior to obtaining the mutational spectra.

15. The method of Claim 12, wherein the mutation detected is present at a mutant fraction about or higher than 10^{-6}.

16. The method of Claim 12, wherein step a) comprises fragmenting the genomic DNA to obtain double-stranded DNA fragments.

17. The method of Claim 16, wherein a double-stranded DNA sample is enriched for one or more target sequences, said enrichment comprising the steps of:

a) denaturing the double-stranded DNA;

b) contacting the denatured DNA with a plurality of probes comprising a sequence complementary to one or more target sequences to form a mixture;

c) maintaining the mixture of step b) under conditions such that probe-fragment hybrid molecules are formed; and

d) isolating the probe-fragment hybrids from the mixture,

resulting in a target-enriched sample and a depleted sample.

18. The method of Claim 17, wherein the probe comprises an affinity moiety unique for a specific target sequence.

19. The method of Claim 18, wherein the isolation of the probe-fragment hybrid is accomplished by contacting the probe-fragment hybrid with a binding partner molecule affixed to a solid support matrix, wherein the binding partner molecule binds to the affinity moiety of the probe.

20. The method of Claim 19, wherein mutant heteroduplexes are subjected to capillary electrophoresis prior to hifiPCR.

21. The method of Claim 19, wherein the enrichment further comprises one or more additional rounds of constant denaturant capillary electrophoresis prior to obtaining the mutational spectra.

22. A method of mutational analysis to detect nuclear gene mutations at mutant fractions at or above 10^{-6} in a target sequence comprising subjecting a DNA sample comprising one or more target sequences to constant denaturant capillary electrophoresis and high fidelity polymerase chain reaction to obtain a mutational

spectrum to detect nuclear gene mutations, wherein, prior to constant denaturant capillary electrophoresis and high fidelity polymerase chain reaction, the DNA sample is enriched for a target sequence and wherein the enrichment comprises two steps wherein the first step comprises a sequence-specific hybridization coupled with a biotin-streptavidin capture system to enrich for DNA molecules comprising the target sequences, and wherein the second step comprises a mutant enrichment using constant denaturant capillary electrophoresis using a wide bore capillary.

23. The method of Claim 22, wherein the double-stranded DNA fragments are enriched for fragments comprising one or more target sequence, said enrichment comprising the steps of:

a) denaturing the double-stranded DNA fragments;

b) contacting the denatured fragments with a probe comprising a sequence complementary to a known target sequence;

c) maintaining the probe and DNA fragments under conditions such that a probe-fragment hybrid molecule is formed;

d) isolating the probe-fragment hybrid; and

e regenerating a double-stranded fragment,

thereby generating an enriched pool of DNA fragments.

24. A method for preparing a plurality of target-enriched DNA samples, wherein each enriched DNA sample comprises one or more particular target sequences, said method comprising the steps of:

a) contacting a DNA sample with a plurality of probes that can hybridize to a plurality of target sequences under conditions suitable for hybridization, wherein each probe that hybridizes to a specific target sequence comprises an affinity moiety wherein the affinity moiety is unique for each target sequence, thereby forming a hybridized sample mixture containing a plurality of affinity-labeled target sequences;

b) contacting the hybridized sample mixture of step a) with a plurality of binding partners to the affinity moieties in step a), wherein each binding partner is attached to a particular solid support matrix and wherein each binding partner binds to a specific affinity moiety; and

c) separating the particular solid support matrices,

thereby separating the particular target sequences, resulting in separate target-enriched DNA samples.

25. The method of Claim 24, wherein the DNA samples are enriched concurrently.

26. The method of Claim 24, wherein at least one paramagnetic solid support and at least one non-magnetic solid support is used.

27. The method of Claim 24,wherein at least one fluorescently-labeled solid support is used.

28. The method of Claim 24, wherein more than one fluorescently-labeled solid support comprising microsphere beads are separated based on fluorescent differences.

29. The method of Claim 24, wherein the DNA sample is fragmented prior to hybridization.

30. The method of Claim 24, wherein mutant enrichment is performed on target-enriched DNA samples.

31. The method of Claim 30, wherein mutant enrichment is performed by constant denaturant capillary electrophoresis.

32. The method of claim 30, wherein mutant enrichment is performed by allele-specific polymerase chain reaction.